THE DESOXYRIBONUCLEIC ACID CONTENT OF ANIMAL CELLS AND ITS EVOLUTIONARY SIGNIFICANCE

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The DNA (desoxyribonucleic acid) content per cell, according to some recent investigations, is a constant for the various somatic cells of an organism, and sperm cells contain one-half this amount per cell (1, 2). The quantity of DNA per cell is a characteristic of each organism. In this work, done independently by two groups of investigators, the DNA per cell was found by determining the quantity of DNA in a suspension containing a known number of cells and then dividing the total DNA by the number of cells. For sperm and erythrocytes the cells themselves were counted; for tissue cells isolated nuclei were prepared, analyzed, and counted.

Knowledge concerning the DNA content per cell has been extended along two lines. On the one hand, more examples have been brought forward. In the original work, for example, the DNA contents of erythrocytes, liver cells, and sperm of fowl were determined and DNA determinations for cells of fowl kidney, spleen, heart, and pancreas have now been added by Davidson and his colleagues (3). These results are given in Table I. In a series of careful measurements by Davison and Osgood on human granulocytes and lymphocytes (from leukemic blood), two quite different cell types, the DNA per cell of the former was found to be 6.25×10^{-9} mg. and that of the latter 5.84×10^{-9} mg. (4). Our own determinations on human sperm gave 2.72×10^{-9} mg. per cell, approximately one-half the value for the somatic cells.

Another line of investigation has been to determine by a cytochemical procedure the DNA content per nucleus (5). In this procedure it is possible to make determinations on single nuclei, either as isolated cells and nuclei or in tissue sections. It has been found that the Feulgen nucleal reaction gives reproducible results and that a microphotometric observation serves as a measure of DNA content. The essential requirement in this procedure is a standard of known DNA content and heretofore none was available. A whole series of standards was provided by the work mentioned above in which DNA per nucleus was determined chemically. One set of standards consisted of nucleated

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erythrocytes with varying DNA contents from a number of different species. Hepatic nuclei from these species made another set of standards. It was shown that under certain conditions the cytochemical Feulgen determinations had the same relative values as the known standards. Under these conditions, then, the *relative* values obtained cytochemically were correct. In this way determinations of DNA content per nucleus were made for some cells for which the values were previously unknown.

In some instances the DNA content per nucleus can at present be determined only cytochemically. There are, for example, in mammalian liver hepatic nuclei which, from measurements of their diameters, have volumes twice, and in other instances, four times that of the smaller hepatic nuclei. Considering the latter to be diploid, the larger nuclei are supposed to be tetraploid and octoploid. It was found that the DNA contents per nucleus of what appear to be diploid, tetraploid, and octoploid nuclei are close to ratios of 1:2:4. This is in accord

TABLE I

DNA Content of Various Nuclei of the Fowl Expressed as Mg. × 10^{-9} per Nucleus

	Erythrocyte	Liver	Kidney	Spleen	Heart	Pancreas	Sperm
Determinations by Mirsky and Ris, 1949	2.34	2.39					1.26
Determinations by Davidson, Leslie, Smellie, and Thomson, 1950	2.49	2.56	2.20	2.54	2.45	2.61	

with previous chemical determinations of DNA content per nucleus in which haploid (sperm) nuclei were found to have one-half the DNA of diploid nuclei.

The Egg Cell.—The DNA content of another haploid nucleus, that of the ovum, has been determined cytochemically. But before this was done, unsuccessful attempts were made to determine by chemical procedures the DNA content of sea urchin eggs using counted numbers of eggs. A value of 6×10^{-8} mg. DNA per cell has been reported for *Arbacia*[1] (6). For the sperm of another sea urchin, *Echinometra*, we found 0.98×10^{-9} mg. DNA per cell. Comparing the two values it would appear as if the sea urchin egg is an exception to the rule that the DNA content per nucleus is a constant for a species. When the DNA for *Echinometra* eggs was determined by essentially the same method which was used for *Arbacia* we found the DNA per cell to be 4.7×10^{-8} mg., apparently fifty times as much as in a sperm cell.

There are several indications that there is not this much DNA in the sea

[1] Dr. Schmidt has informed us that the value given in his paper, 6×10^{-6} mg. DNA, is incorrect. Using the diphenylamine reaction, C. and R. Vendrely (7) have reported a value of 0.22×10^{-6} mg. DNA concerning which they express skepticism.

urchin nucleus. This can be seen at once by considering the size of the egg nucleus, the quantity of DNA it is purported to contain, and the fact that it is Feulgen-negative. After fixation, preparatory to carrying out the Feulgen reaction, the nucleus of the *Echinometra* egg is 9.3 μ in diameter, which, assuming a specific gravity of unity, would be equivalent to a mass of 4.2×10^{-7} mg. The mass of a sperm nucleus of *Echinometra* is about 0.16×10^{-7} mg. This value was found by drying a counted suspension of sperm nuclei, dividing the total weight by the number of sperm to get the dry weight of a single nucleus, and then multiplying by 5 to get the wet weight. The sperm nucleus contains 0.98×10^{-9} mg. DNA and if the egg nucleus were actually to contain 4.7×10^{-9} mg. DNA, the latter would be intensely Feulgen-positive, as the sperm nucleus is. Since the egg nucleus is, in fact, Feulgen-negative, this direct test shows that it contains very much less DNA than has been supposed. If, on the other hand, the egg does indeed contain the same quantity of DNA as is found in the sperm, then its nucleus would be Feulgen-negative, and to determine chemically this amount of DNA would mean determining one part of DNA in as much as 10,000 parts of egg material for the dry egg cell weighs 0.7×10^{-4} mg. This cannot be accomplished by present methods. Furthermore, we have examined two of three methods (the diphenylamine and the Schmidt-Thanhauser (8)) in their application to sea urchin eggs and found that, due to interference by materials of unknown composition, far too high values for DNA are obtained.

To compare the relative DNA contents of egg and sperm nuclei we have resorted to the cytochemical Feulgen procedure. For this purpose it is necessary to choose an egg nucleus with a sufficiently high concentration of DNA to be Feulgen-positive. The egg of *Ascaris megalocephala* has a small, Feulgen-positive nucleus. Following van Beneden's classical observations, the egg nucleus can best be compared with the sperm nucleus after fertilization, when the sperm nucleus has already penetrated into the egg and has enlarged, just before fusing with the egg nucleus, at a time when the two nuclei are of the same size and also have the same structure. A Feulgen preparation made at this time shows the two nuclei indistinguishable from each other; the two haploid nuclei, therefore, have identical quantities of DNA.

The data now available provide considerable support for the rule that in the cells of an organism there is a characteristic and constant quantity of DNA for each haploid set of chromosomes. Within what limits this rule holds is not yet known.[2] It should be recalled that until recently it was generally supposed by cytologists that DNA contents of different nuclei of the same organism are highly variable. "The nucleic acid charge" was considered to be one of the most

[2] Recent unpublished determinations by Dr. Hewson Swift on individual Feulgen-stained nuclei of two plants—corn and *Tradescantia*—"support the view that DNA occurs in well-marked units characteristic of the strain or species."

important *variable* characteristics of a cell. This point of view depended upon innumerable observations of differences in intensity of staining. If the total quantity of DNA in the nucleus is considered, differences in intensity can of course be compensated for by differences in nuclear volume. This is obvious as soon as one measures the *total* DNA of a nucleus, and this cytologists have failed to do, at least in any systematic way, until recently. In all probability, there now will be many determinations of total nuclear DNA content, so that within what limits constancy holds should soon be known.

Further investigation is needed of alleged variations in DNA content per cell in somatic cells of mammals. In cattle, for example, it has been reported that whereas the DNA per cell of calf liver cells is 6.2×10^{-9} mg., it is 8.4×10^{-9} mg. for beef liver cells, and the latter value is not due to polypoidy (2). Whether this difference is due to the age of the animal, or simply to experimental error is not yet known. For the rat it has been reported that liver cells contain 6×10^{-9} mg. DNA per cell (1). More recent experiments give the same result, although in some older rats of the Wistar strain, a value of 8×10^{-9} mg. DNA was obtained for liver cells, the higher value being attributed to polypoidy (9). The higher value, 8×10^{-9} mg. for cells of both liver and kidney of the rat, was also found in four different experiments by Mirsky and Kurnick (10). In these experiments the effect of fasting was studied. It was found that in livers which had lost as much as half of their weight, the DNA content per nucleus remained unchanged. This interesting example of constancy in DNA content was found in 1947 at the very beginning of our work on the DNA contents of nuclei. Similar results have since been published by Mandel (11).

DNA Content in Relation to Evolution.—When the constancy of DNA content for each haploid set of chromosomes was first recognized, it was also realized that this value, varying from one organism to another, is a quantitative characteristic of the germinal material of an organism. A survey of a few invertebrates and of a wider range of vertebrates was accordingly undertaken, and the results are here presented. They are considered from an evolutionary standpoint partly because changes in the DNA are changes in the germinal material, and changes in this material have a special significance in evolution.

All DNA determinations were made by the Schmidt-Thanhauser method (8) on counted cell suspensions. The need to count cells limits the material that can be used to sperm cells and nucleated erythrocytes. The cells of sponges are an exception for if the sponge is pressed through fine gauze and suspended in calcium-free sea water, the cells are separated and thus easily counted. For determinations of DNA in sponge cells both the Schmidt-Thanhauser method (8) and the diphenylamine reaction, as described by Schneider (12), were used. Before the determination the cells were dried. This was done by centrifuging a given volume of counted cells. The residue was washed with 66 per cent alcohol to remove salts, then with 95 per cent alcohol, and finally with ether.

While the ether is being removed from the final residue by immersing in warm water, the sediment is rubbed so that a fine powder is obtained. This powder can be dried to constant weight in 10 minutes at 106°C. Known volumes of counted suspensions of washed erythrocytes were dried in the same way. Some hemolysis of teleost erythrocytes frequently occurred when they were washed, but this rarely happened with erythrocytes of other vertebrates, and the dry weight per cell is accordingly given in Table IV.

Sperm cells were washed with 1 per cent citric acid to remove tails and mid-pieces, and microscopic examination showed that in this way clean sperm heads were obtained. Sperm cells were counted, before the citric acid treatment, in sea water to which a little phenol was added to immobilize the sperm. The sperm heads were dehydrated with 95 per cent alcohol, ether, and then dried. In some cases so little sperm was available that it could not be dried and weighed; after counting the sperm the material was centrifuged and DNA determined in the sediment.

In Table II are given the DNA contents of the cells of two species of sponge and of the sperm cells of twelve other invertebrates. In Tables III and IV are given the DNA contents per cell for the erythrocytes of many vertebrates. The values range from 0.11×10^{-9} mg. for a diploid sponge cell to 168×10^{-9} for a diploid cell of amphiuma, a urodele—a 1500-fold increase.

DNA in Cells of Invertebrates.—Among invertebrates the lowest values are among the most primitive animals, lower values being found for sponges and coelenterates than for echinoderms and crustaceans, and molluscs. And among the molluscs the more primitive members have the lowest DNA content. The squid is a far more highly developed animal than are the limpet, snail and chiton; and the squid has far more DNA per sperm than is found in the lower molluscs. Our knowledge of DNA per cell in invertebrates is, however, so scanty that formulation of a rule is hardly warranted.

DNA in Cells of Vertebrates.—For cells of vertebrates our information is more extensive. In the vertebrates DNA per cell does not increase in the more highly developed animals; there is, indeed, as will be seen later, evidence that in some instances DNA per cell decreases in the course of evolution. Paleontological knowledge concerning vertebrate evolution is given in Romer's Vertebrate Paleontology (13).

Fishes.—A first glance at the data (Table III) of DNA per cell for the teleostean fishes may give the impression that for these animals our data are extensive because some thirty species have been studied. There are, however, some 20,000 living species, and their variety is so great that classification has been an exceedingly perplexing problem. Although there is much doubt among systematists about how natural some of the higher taxonomical categories are, there is agreement that the families represent natural groupings. With this in mind, if one looks at the data for DNA per cell for teleosts, two points are

obvious; first, there is on the whole great variety; and second, within families there is considerable uniformity. Uniformity within the family is striking because in many cases the different species included in a family show marked superficial differences. Among the Carangidae, for example, there is a vast

TABLE II

DNA Content per Nucleus of Some Invertebrates

Animal	Type of cell	Weight of cell in sponges. In others weight of sperm head	DNA per nucleus mg. $\times 10^{-9}$
Sponges:			
Tube sponge	Diploid	1.04×10^{-8} mg.	0.12
Orange sponge, *Dysidea crawshagi*	Diploid	1.13×10^{-8} mg.	0.11
Coelenterate:			
Jellyfish, *Cassiopeia*	Sperm, haploid	1.43×10^{-9} mg.	0.33
Echinoderms:			
Sea urchin, *Echinometra*	" "	3.23×10^{-9} mg.	0.98
Sea urchin, *Lytechinus*	" "	3.50×10^{-9} mg.	0.90
Sea cucumber, *Stichopus diabole*	" "		0.99
Annelid:			
Nereid worm	" "		1.45
Molluscs:			
Limpet, *Fissurella barbadensis*	" "	1.71×10^{-9} mg.	0.50
Snail, *Tectarius muricatus L.*	" "	3.54×10^{-9} mg.	0.67
Chiton tuberculatus	" "	2.2×10^{-9} mg.	0.63
Squid	" "		4.5
Crustaceans:			
Cliff crab, *Plagusia depressa*	" "		1.49
Goose barnacle	" "		1.46
Tunicate:			
Asidia atra	" "	1.06×10^{-9} mg.	0.158

difference in size between the round robin and the bonito; and in the Mugilidae-Sphyraenidae the mullet and barracuda at first seem like quite different fish.

Little indication concerning the evolutionary significance of DNA content per cell is given by the teleostean data. The Teleostei were derived from the Holostei, whose ancestors in turn were the Chondrostei. Among the most primitive Teleostei, those who have departed least from their holostean ancestors are the Clupeidae, the herrings. The most recent teleosts, on the other hand, are the spiny teleosts, the Acanthopterygii, among whom are fish of

many families which we have studied, such as the Balistidae and Carangidae. There are a few surviving species of the Holostei and of the still more ancient

TABLE III

DNA Content of Erythrocytes of Fishes Expressed as Mg. × 10^{-9} per Cell

Agnatha:	
Lamprey, *Petromyzon*	5
Elasmobranchii:	
Shark, *Carcharias obscurus*	5.46
Shark, *Carcharias longimanus*	6.67
Chondrostei:	
Sturgeon, *Acipenser sturio*	3.2
Holostei:	
Bowfin, *Amia*	2.3
Teleostei:	
Clupeidae	
Pilchard, *Harengala sardinia*	2.04
Shad, *Alosa*	1.97
Cyprinidae	
Carp	3.49
Siluridae	
Catfish, *Ameiurus nebulosus*	1.89
Scaridae	
Rainbow parrot, *Pseudoscarus guacamaia*	2.5
Red tailed parrot, *Sparisoma brachiale*	2.45
Mudbelly, *Scarus croicensis*	2.58
Balistidae	
Triggerfish, *Balistes capriscus*	1.07
Carangidae	
Bonito, *Zonichthys falcatus*	1.35
Jack, *Caranx latus*	1.21
Round robin, *Decapterus punctatus*	1.32
Acanthuridae	
Doctor fish, *Acanthurus hepatus*	1.38
Teleostei (*continued*)	
Ostraciontidae	
Cow fish, *Lactophrys quadricornis*	1.91
Aulostomatidae	
Trumpet fish, *Aulostomus maculatus*	1.39
Holocentridae	
Squirrel fish, *Holocentrus ascensionis*	1.31
Mugilidae—Sphyraenidae	
Mullet, *Mugil curema*	1.38
Barracuda, *Sphyraena barracuda*	1.37
Sparidae	
Sheepshead porgy, *Calamus calamus*	2.24
Bream, *Diplodus argenteus*	1.61
Haemulidae	
Blue striped grunt, *Haemulon sciurus*	1.20
Yellow grunt, *Haemulon flavilineatum*	1.33
Gerridae	
Shad, *Eucinostomus gula*	0.94
Lutianidae	
Yellow tail, *Ocyurus chrysurus*	2.1
Gray snapper, *Lutianus griseus*	2.1
Silk snapper, *Lutianus hastingsi*	2.1
Belonidae	
Hound fish, *Tylosurus acus*	2.2
Serranidae	
Red hind, *Epinephelus guttatus*	2.09
Butterfish, *Cephalopholis fulvus*	1.93
Hamlet, *Epinephelus striatus*	2.05
Gag, *Mycteroperca tigris*	2.2
Monkey rockfish, *Trisotropis venenosus*	1.83

Chondrostei. Of the former, *Amia*, the bowfin, and of the latter, *Acipenser*, the sturgeon, were available to us. Examination of the data for all the fish that have just been mentioned shows no obvious evolutionary trend. It is perhaps noteworthy that some of the most recent teleosts have lower values than are

found in older forms. To say that there has been a drop in DNA per cell would, however, imply that the older forms actually examined are themselves the ancestors of the more recent forms. This implication would be unwarranted because the lines of teleost descent are not clearly defined. Furthermore, a living survivor of a primitive group may well have itself changed in the course of time so that it is no longer representative of what was indeed an ancestral group. If this were not possible, the lamprey would be of the greatest interest for the present work, for it is now regarded as a survivor of the jawless vertebrates, the most ancient of known vertebrates. The DNA content per cell in the lamprey is considerably higher than that of most of the teleosts which we have examined.

Dipnoans, Amphibians, Reptiles, and Birds.—The teleosts form one great branch of the vertebrates; on another branch are the amphibians, reptiles, birds, and mammals. In each of these classes determinations of DNA contents per cell of a number of species have been made (Table IV), perhaps enough to permit some generalization about the class as a whole. Our purpose in doing this is to attempt to estimate the DNA content per cell of extinct forms which were the ancestors of living groups. Consider the reptiles, for example. Of the vast assemblage of reptiles which flourished in Mesozoic times, only three orders survive (if we omit *Sphenodon*): crocodiles and alligators, snakes and lizards, and turtles. These three orders have all diverged, each in its own way, from the base of the reptilian family tree. The fossil record shows that the turtles have diverged little and the crocodiles much. Paleontologists consider that the birds are derived from reptiles who were fairly close relatives of the alligators and crocodiles, and that, on the other hand, mammals are descended from reptiles who were close to the base of the reptilian family tree. The DNA content per cell of the turtle (5.1×10^{-9}) is just a little below that of mammals (5.5 to 6.0×10^{-9} mg.), indicating that in the evolution of mammals from reptiles there was little change, possibly a slight increase in DNA content per cell. Since the DNA content per cell of the alligator (5.0×10^{-9} mg.) is nearly twice that of birds (2.3 to 2.9×10^{-9} mg.) there was a considerable decrease in the value during the evolution of birds from reptiles. The main uncertainty in these conclusions concerns the DNA content per cell of the reptilian ancestors of mammals and birds. This uncertainty is diminished by the fact that such phylogenetically different reptiles as the turtle and alligator have practically the same DNA content per cell, for this means that the generality of extinct reptiles probably had the same value. It should be noted that among the snakes, which are the newest of reptilian groups, there is a tendency for the DNA content per cell to decline: common water snake, 5.02×10^{-9} mg.; pilot black snake, 4.28, and black racer, 2.85.

It is generally agreed by paleontologists that reptiles were derived from amphibians. There is evidence that in this process the DNA content per cell

decreased. Of the many orders of amphibians that once existed, only a few have survived. The main living amphibians are the Anura (frogs and toads) and the Urodela (salamanders and newts). If values for DNA content per cell

TABLE IV

DNA Content and Mass of Erythrocytes of Various Vertebrates. DNA Expressed as Mg. × 10^{-9} per Cell and Mass as Mg. × 10^{-8} per Cell

Animal	DNA	Mass
Dipnoan:		
African lungfish, *Protopterus*	100	161
Amphibians:		
Amphiuma	168	368
Necturus	48.4	40.5
Frog	15.0	27
Toad	7.33	13.7
Reptiles:		
Green turtle	5.27	18.4
Wood turtle	4.92	14.1
Snapping turtle	4.97	
Alligator	4.98	14.9
Water snake	5.02	13.7
Pilot snake	4.28	13.3
Black racer snake	2.85	10.2
Birds:		
Domestic fowl	2.34	4.39
Guinea hen	2.27	4.58
Duck	2.65	5.44
Goose	2.92	7.37
Mammals:		
Man—Lymphocytes	5.84	
Granulocytes	6.25	
(Data of Davison and Osgood)		
Rat—lymphocytes	6.1	
(Data of Cunningham, Griffin, and Murray)		

of amphibians and reptiles are compared, the latter are much lower. The values for diploid cells are (all in mg. × 10^{-9}): frog, 15; toad, 7; *Necturus*, 48; amphiuma, 168; reptiles close to 5. It is hazardous to infer simply from the DNA contents of the Anura and Urodela what the DNA content per cell was of the amphibian ancestors of the reptiles.

For information about these extinct amphibians a glance at the ancestors of

the amphibians should be of some value. The ancestors of the amphibia were the crossopterygian fishes. (The Crossopterygii are distinctly different from the Chondrostei, the ancestors of the teleost fish.) Until recently it was supposed that the Crossopterygii have been extinct for ages, but a few years ago a single surviving specimen, *Latimeria*, was discovered. The finding of another specimen is still being awaited by many biologists. There are available, however, three well known surviving species of a group of fishes which were very closely related to the early Crossopterygii. These are the lung fishes, the Dipnoi. A knowledge of the DNA content per cell of lung fishes would have some bearing on the DNA of primitive amphibians and thus on whether there was a decline in DNA per cell in the evolution of reptiles from amphibians.

The three surviving lung fish are *Epiceratodus* in Australia, *Protopterus* in Africa, and *Lepidosiren* in South America. Of the three, the first is very close to an extinct form and in a different group from the latter two. Since a specimen of *Protopterus* was available to us, its DNA content per cell was determined and found to be 100×10^{-9} mg. Although the other species were not available, the size of their nuclei shows that their DNA contents are much the same as that of *Protopterus*. In the course of this work nuclear sizes and DNA contents of many erythrocytes have been measured and a close correlation between them has been found. The erythrocyte nucleus of *Protopterus* is 22×12 μ, that of *Lepidosiren* 14×8 μ (14), and that of *Epiceratodus* 16×12 μ. (This was kindly measured for us by Dr. and Mrs. I. M. MacKerras of Brisbane, Australia.) Since the different living lung fish have about the same DNA contents per cell, it is probable that a value of the same order was present in the extinct lung fish and in their close relatives, the crossopterygian ancestors of the amphibians. The extremely high dipnoan DNA content per cell is similar to that found in urodeles and decidedly different from what has been found for any other vertebrate. It may be said, therefore, that the ancestors of the amphibia and the amphibian ancestors of the reptiles probably had far higher DNA contents per cell than did the reptiles; that over a long period of vertebrate evolution there probably was a decline in DNA per cell.

DNA is part of the germinal material. What changes in the nature of the germinal material are associated with the large differences in DNA content per cell observed in vertebrates? Comparing the largest and one of the smallest examples among vertebrates, one finds that a cell of amphiuma, a urodele, contains 70 times as much DNA as is found in a cell of the domestic fowl, a far more highly developed animal. It seems most unlikely that amphiuma contains 70 times as many different genes as does the fowl or that a gene of amphiuma contains 70 times as much DNA as does one in the fowl. To make a somewhat different comparison: a cell of amphiuma contains 170 times as much DNA as does a cell of a relatively closely related animal, the trigger fish, whereas a cell of the latter contains only nine times as much DNA as does a cell of a sponge, which is far removed phylogenetically from any vertebrate. The vari-

ations in DNA content per cell in vertebrates would hardly seem to be due simply to difference in the number of genes. Perhaps variations in DNA per cell are associated with differences in the number of strands in the chromosomes. According to this view, where polypoidy is not a factor and where enormous variations in numbers of different genes seem unlikely, DNA content may be some indication of the number of strands in a chromosome.

In vertebrates there does not appear to be a simple relationship between quantity of DNA per cell and the number of different genes. It seems possible, however, that in some primitive organisms the number of DNA molecules represents the number of genes. In a haploid sponge cell there are 40,000 molecules of DNA, if a molecular weight of a million is assumed. But if in each chromosome of a vertebrate there are many strands containing DNA, the same may be true in invertebrates. There may therefore be more than one DNA molecule for each gene, even in the sponge.

The relationship between DNA and the size or number of genes is obscure, but the relationship between the DNA content of a cell and the size of the cell is clear: in general, when homologous cells are compared the greater the DNA content, the larger the cell. In the nucleated red cells of vertebrates, a series of homologous cells, there is an approximately direct relationship between cell mass and DNA content, and considering the physiological variations in quantity of hemoglobin per cell, no more than an approximate relationship would be expected. Of all the diploid cells which we have examined the sponge cell weighs the least, and it also contains the smallest amount of DNA. In the course of evolution there have been great changes, both increases and decreases, in cell size and in DNA content.

A relationship between DNA content and cell size is but another aspect of the relationship between number of sets of chromosomes and cell size. The classical experiments on the subject are those of Boveri (15). In experiments on sea urchin eggs he was able to vary the number of chromosomes in several different ways, and in every case cell size was found to depend upon the number of chromosomes present. When in different animals DNA per cell varies, it does not mean, of course, that there is a variation at the same time in chromosome number. What can be said, however, is that when DNA per cell increases, whether due to an increase in number of chromosomes or to an increase in the number of strands per chromosome, an increase in cell size follows.

SUMMARY

1. Evidence is summarized for the constancy of DNA content for each set of chromosomes in the various cells of an organism.

2. The DNA contents of the egg and sperm nuclei are the same.

3. A brief survey is given of DNA contents per cell in invertebrates and vertebrates.

(*a*) In invertebrates there is some slight evidence that when primitive and

higher forms are compared the amount of DNA per cell is increased in the latter.

(*b*) In fishes there is a tendency for the amount of DNA per cell to remain constant within the different species of a family.

(*c*) The values of DNA per cell in lung fishes, amphibians, reptiles, and birds suggest that in the evolution of these vertebrates there has been a decline in DNA content per cell.

4. Concerning the significance of quantity of DNA per cell in vertebrates:

(*a*) It appears not to be in proportion to the number of different genes in a cell.

(*b*) It may be related to the number of strands in the chromosomes.

(*c*) In homologous cells of different animals it is directly related to the mass of the cell.

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